1* Comparison of bioinformatics, reporter minigene and mRNA study to analyse the effect of unclassified variants on *CFTR* mRNA splicing

M.-P. Audrézet[1], C. Ka[1], K. Le Millier[1], M. Planes[1], G. Le Gac[1], C. Férec[1]. [1]*CHRU de Brest, Laboratoire de Genetique Moleculaire, Brest, France*

The *CFTR* gene is one of the most highly mutated human genes with more than 1600 different mutated alleles reported, and besides the most frequent mutations, the deleterious effect of many variations remains unclear, which sometimes confuse molecular geneticists, particularly for intronic or exonic silent variations, which can affect mRNA splicing.
Several softwares have been developed for *in silico* prediction of the impact of sequence variation on splicing, and they are commonly used in molecular laboratories. But even if they have proven their effectiveness for many genes, it is important to confirm by *in vitro* functional analysis.
The aim of our study is to compare the bioinformatic predictions to results obtained with a reporter minigene and by direct analysis of mRNA from patients.
On the basis of the bioinformatic predictions, more than 50 variations have been classified into four classes according to the expected effect on mRNA splicing (complete or partial exon exclusion, cryptic alternative splicing site, or no effect). Two variations from each class were tested in our minigene vector and at the mRNA level.
As expected, mutations affecting consensus donor splice lead to complete exon exclusion and frequent polymorphisms located in the last nucleotides of exons did not affect mRNA splicing.
More interestingly, some mutations, initially classified as splice mutations because of their location on the acceptor sites yielded an unexpected result in bioinformatics predictions, which was confirmed by minigene and mRNA analysis.
This reporter minigene study will be extended to the 50 variations selected, and in cases of discrepancy, analysis of mRNA will be performed.

2 Detection of aberrant transcripts by *CFTR* mRNA analysis

M.D. Ramos[1], J. Gimenez[1], A. Alvarez[2], J. de Gracia[2], T. Casals[1,3]. [1]*IDIBELL, Center for Molecular Genetic Diagnosis, Barcelona, Spain;* [2]*Hospital Vall d'Hebron, Cystic Fibrosis Unit, Barcelona, Spain;* [3]*IDIBELL, Human Molecular Genetics Group, Barcelona, Spain*

The mRNA analysis has evidenced to be an efficient tool to detect mutations disrupting the normal splicing process in genetic disorders. So far, *CFTR* gene scanning and the large rearrangements study have allowed us to raise a detection rate around 97% in the CF Spanish population. Currently, we have evaluated the systematic analysis of *CFTR* RNA to improve this percentage. For this purpose we have collected nasal epithelial cells from patients and controls and we have developed five PCRs to cover the complete RNA using primers already described and/or specifically designed (Primer3 v.0.4.0). The fragments size for a normal transcript ranges from 600 bp to 1100 bp. In this preliminary study we have included splicing mutations and large rearrangements leading to known aberrant transcripts as well as missense mutations potentially involved in a reduction of the wild type *CFTR* transcripts. *In silico* analysis has been also performed with the Alamut® software v.1.5 to support the pathogenic effect of the mutations. Once the protocol conditions have been optimized we are carrying out the RNA analysis in two different groups, (a) CF samples partial/total uncharacterized to identify the unknown mutations; and (b) sequence variants found in our population to assess their pathologic effect. We believe the implementation of the RNA analysis will contribute to identify novel molecular defects and mechanisms concerning the wide spectrum of the CF. Results will be discussed.
Supported by the ISCiii (PI080041) and Fundación Sira Carrasco

3 DHPLC analysis of CFTR gene: reasons for request and results

A. Ravani[1], M. Taddei Masieri[1], A. Venturoli[1], P. Rimessi[1], C. Trabanelli[1], S. Carturan[1], B. Dolcini[1], S. Brioschi[1], F. Gualandi[1], A. Ferlini[1]. [1]*Azienda Ospedaliera Universitaria "S. Anna", Laboratorio Genetica Molecolare – U.O. Genetica Medica, Ferrara, Italy*

The great allelic heterogeneity in Cystic Fibrosis encourages the diagnostic application of methods for the rapid screening of mutations.
In our Lab we have adopted DHPLC as enlarged mutation screening method, following the search of the most frequent molecular defects.
Based on our experience, the reasons for the request of this deeper investigation are:

1. CF Clinical diagnosis, typical or atypical, where only one or none mutations have been identified by searching the most frequent molecular defects.
2. Male infertility, with suspicion of CBAVD
3. Family History of Cystic Fibrosis, without molecular characterization of the proband
4. Partner heterozygous for a pathogenetic mutation of the CFTR gene.

The last group constitutes more than 75% of the requests we currently receive for DHPLC from many regional and national Centres.
Since the end of 2006, we have analyzed 481 subjects, of which 376 with partner carrier of Cyctic Fibrosis.
Of them, 41 were heterozygous for a rare pathogenetic mutation, with a frequency of about 10%, three times higher than in general population.
Also in some cases of echogenic bowel, when the first analysis revealed one of the partner as a carrier of Cystic Fibrosis, we performed DHPLC in the second one (if the gestational age allowed it). We have analyzed 124 couples with hyperechogenic bowel: 6 were constituted by two CF carriers (4 of them identified by DHPLC). The subsequent investigation on foetal DNA confirmed the status of compound heterozygous for two CFTR mutations, compatible with the clinical diagnosis of Cystic Fibrosis.

4 Simple and accurate assays for targeting *CFTR* mutations of specific geographic/ethnic origins by PCR allelic discrimination

C. Vasseur[1], A. LeFloch[1], B. Costes[1], A. de Becdelièvre[1], T. Casals[2], C. Costa[1], M. Goossens[1], E. Girodon[1]. [1]*Hôpital Henri Mondor, Genetics, Creteil, France;* [2]*IDIBELL, Human Molecular Genetics, Barcelona, Spain*

Search for CF mutations of frequency >1% according to geographic/ethnic origins of individuals is a European recommendation (Dequeker et al., Hum Mutat 2009). Given heterogeneity of populations living in European countries, additional search for mutations specific of ethnicities by using sequencing, beside the use of a common kit, is time-consuming and regularly leads to identification of unknown variants. Such variants make genetic counselling delicate, in particular when found in CF carriers' partners and cases of foetal bowel anomalies. There was thus a need for tests focused on specific mutations.
We developed simple assays targeted on 6 geographic origins (Italy, Spain, Portugal, Turkey, North Africa and Black Africa), containing 3–8 mutations and based on probe hydrolysis during PCR and fluorescence detection (custom TaqMan® SNP genotyping). Homozygous and heterozygous mutant controls were generated from cloned PCR products for each mutation. Validation was made on blind series of DNA representative of different genotypes and on prospective series of patients' DNAs to be tested in routine analysis, in comparison with sequencing.
The results fulfilled the validation requirements in terms of sensitivity, specificity, repeatability, robustness and measurement uncertainty. These assays are simple and rapid to implement in routine practice and simplify genetic counselling.